IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Paula M. Olhoft et al.	Examiner: Stuart F Baum
Serial No.:	10/813,482	Group Art Unit: 1638
Filed:	March 30, 2004	Docket No.: 600.479US2
Title:	METHOD TO ENHANCE AGROBACTERIUM-MEDIATED TRANSFORMATION OF PLANTS	

RULE 132 DECLARATION

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Dr. Paula Olhoft, declare and say as follows:

1. I am one of the named co-inventors of subject matter claimed in the above-identified application, Serial No. 10/813,842, filed on March 30, 2004, which is a continuing application of Serial No. 09/738,398, filed on December 15, 2000, now U.S. Patent No. 6,759,573, which claims the benefit of the filing date of Serial No. 60/170,981, filed on December 15, 1999, and make this Declaration in support of the patentability of the claims in the above-identified application.

2. In the Office Action dated October 4, 2007 for the above-referenced matter, the Examiner rejected claims 58-66, 68 and 71-74 under 35 U.S.C. § 102(b) as being anticipated by Perl et al. (Biotechnology, 14:624 (1996)) and rejected claims 57-60, 62-67, 71-73, and 75-76 under 35 U.S.C. § 103(a) as being unpatentable over Enrique-Obregón et al. (Biotechnologia Aplicada, 14:169 (1997)).

3. Perl et al. tested the effect of six agents (which included cysteine and DTT) in solid media on co-cultures of *Agrobacterium* and embryogenic grape callus (treatments 1-6 in Table 1 in Perl et al.). Perl et al. report that the presence of cysteine in the co-cultivation media did not reduce necrogenesis while the presence of DTT in the co-cultivation media inhibited grape callus browning (page 625).

4. Figure 3 in Perl et al. discloses the method which was employed to prepare stable transformed grape plants. Specifically, Perl et al. co-cultivated *Agrobacterium* and embryogenic grape callus for 2 days on a solid media having polyvinylpolypyrrolidone (PVPP). After that, the callus was transferred to a double layer medium with PVPP in the solid media and DTT in the upper (liquid) layer for 7 days, followed by transfer to selective media. Perl et al. disclose that the combination of PVPP and DTT was found to improve plant viability, and that those agents inhibited tissue necrosis but did not affect *Agrobacterium* virulence.

5. The combination of PVPP and DTT in the double layer medium was likely chosen in experiments to produce stable transformants because other combinations, including those with cysteine or DTT in the solid or liquid media of double layer media, did not block necrogenesis (treatments 7 and 9-12 in Table 1 in Perl et al.)

6. The 2 day co-cultivation step in Perl et al. allows for *Agrobacterium* infection of the callus, and the 7 day culture step in Perl et al. is intended to favor callus recovery and growth prior to selective pressure. Thus, while two sulfhydryl containing agents were tested in solid co-cultivation media in Perl et al. (treatments 3 and 4 in Table 1), neither agent was chosen for the co-cultivation step in a protocol to prepare stable transformants.

7. In contrast, the claims in the present application are directed to the use of one or more sulfhydryl containing agents during co-cultivation in an amount effective to enhance the stable transformation of plant tissue or cells with *Agrobacterium*.

8. Moreover, there is nothing in Perl et al. that would motivate one of skill in the art to add one or more sulfhydryl containing agents during co-cultivation of plant cells and *Agrobacterium*, particularly in view of the disclosure in Perl et al. that cysteine in the co-cultivation medium did not inhibit necrogenesis and that PVPP, not cysteine or DTT, was selected as the agent in the co-cultivation medium in a protocol to prepare stable transformants.

9. Enriquez-Obregón et al. report on the effect of a combination of ascorbic acid, cysteine and silver nitrate in pre-coculture liquid medium, co-culture medium, or both, on *Agrobacterium*-mediated transformation in sugarcane (Table 3). Cysteine was employed individually at 40 mg/L or 90 mg/L in experiments to determine percent explant viability (Table 2) or in the combination at 40 mg/L to determine percent explant viability (Table 2) or to determine percent GUS positive explants, percent herbicide resistance or the presence of a transferred gene after *Agrobacterium*-mediated transformation (Table 3)

10. Unhealthy plant cells often do not withstand *Agrobacterium*-mediated infection or selective pressures (even if the cells have a non-native gene that encodes resistance to the selective agent). When any agent is added to plant media in an effort to improve outcome, there is a balance between plant cell viability and agent toxicity. See, for example, the explant viability data in the presence of certain concentrations of silver nitrate, cysteine or ascorbic acid in Table 2 in Enriquez-Obregón et al. (none of the treatments result in 100% viability). Moreover, at one concentration, the agent may not substantially impact plant cell viability while at a higher concentration the agent may decrease plant cell viability. Note that for experiments to test the effect of the combination of agents on *Agrobacterium*-mediated gene transfer, Enriquez-Obregón et al. selected the lower concentration of the two tested concentrations of each of the three agent to include in pre-coculture liquid medium and co-culture medium (Table 3).

11. In view of the balance between plant cell viability and agent toxicity, and *Agrobacterium*-mediated virulence, there would be no reason to try higher amounts of any of the agents in the combination disclosed in Enriquez-Obregón et al. because of potential cytotoxicity.

12. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 3/25/2008 By: _____
Dr. Paula Olhoft